mitochondria CRC (287 ± 17 *vs.* 180 ± 12 nM/mg mitochondrial protein, $p<0.05$), but it had no effect on CRC from mitochondria of GPER-/- hearts (170 ± 33 *vs.* 167 ± 7 nM/mg mitochondrial protein). These results conclusively demonstrate that GPER plays an important role in mediating rapid E2-induced cardioprotection after ischemia/reperfusion. Supported by NIH.

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Validation of Reperfusion Duration for Myocardial Infarction Assessment in the Langendorff Ischemia Model of Isolated Mouse Heart

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Introduction: Perfused isolated heart is a widely used *ex-vivo* organ model as it allows rigorous control of parameters what is not readily achievable by an *in vivo* model. However, there is disagreement on the reperfusion duration to assess the myocardial infarction by TTC staining. Many authors have used long reperfusion times (~120 min) in conditions where the hearts during ischemia were suspended in air water-jacketed chamber maintained at37°C. Others have determined myocardial infarction with short reperfusion durations (30-40min) where the hearts were submerged in a normothermic physiological solution. In this work where hearts were immersed in Krebs Henseleit (KH) solution during ischemia we demonstrated that a reperfusion duration of 40 min is adequate to determine infarct size after ischemia as longer reperfusion times of 60 and 90 min showed the same maximal infarct size.

Methods and Result: We compared different reperfusion durations (40, 60 and 90 min) of isolated mice hearts immersed in KH buffer after 18 min of normothermic ischemia. Hearts were perfused using Langendorff apparatus with KH buffer oxygenated with 95% O_2 + 5% CO_2 at37°C. The heart function was recorded and myocardial infarction assessed by TTC staining at the end of reperfusion. Our data show no significant difference between the groups reperfused for 40, 60 or 90 min in infarct size and heart function recovery.

Conclusion: These results indicate that when the isolated mice hearts are immersed in a normothermic physiological solution during ischemia, reperfusion for 40 min is sufficient to reach the maximum infarction.

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Ivabradine and Mechanical Unloading Increase Sarcoplasmic Reticulum Calcium Content in a Rodent Model of Heart Failure

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Improvements in Ca2+ handling, and sarcoplasmic reticulum (SR) Ca2+ regulation in particular, are associated with myocardial recovery in patients with end stage heart failure (HF) treated with left ventricular assist devices (LVAD). Ivabradine (IV), a novel heart rate reducing agent has been shown to have positive Ca^{2+} regulatory effects in rodent HF, and is used in human HF therapy; however, the effects of IV during LVAD treatment have not been studied. We studied the chronic effects of IV on cellular Ca^{2+} handling during mechanical unloading (MU) of HF hearts. HF was induced by left coronary artery ligation and was characterized by reduced ejection fraction (EF), smaller Ca^{2+} transient amplitude (CaT) and reduced SR Ca^{2+} content compared to age matched sham operated animals (SH). MU (4 weeks) was achieved by heterotopic abdominal heart transplantation and IV (10mg/kg/day) was administered for 4 weeks. Isolated ventricular myocytes were loaded with Indo-1 AM and field stimulated at 1 Hz.

CaT was significantly elevated in the MU+IV group (indo-1 ratio units): MU+IV: 0.25 ± 0.06 (n=45) $p< 0.001$ vs MU: 0.18 ± 0.05 (n=40), and $p<0.001$ vs SH: 0.17 ± 0.05 (n=45)). IV augmented SR Ca^{2+} content in combination with MU compared with MU alone and to a level even greater than SH: SR Ca^{2+} content (indo-1 ratio units): MU+IV: 0.27 ± 0.07 (n=45) $p< 0.05$ vs MU: 0.24 ± 0.05 (n=40), and $p<0.001$ vs SH: 0.22 ± 0.07 (n=45))

Our results show that IV treatment combined with mechanical unloading induces a larger increase of Ca^{2+} transient amplitude and SR Ca^{2+} content compared with mechanical unloading alone. These effects may be exploited to enhance myocardial recovery in patients receiving LVAD therapy for HF.

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Bioenergetic Supply and Demand in the Cardiomyocyte

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The mechanisms that regulate the control of energy demand and supply in the myocardium are crucial for maintaining normal cardiac function. Although a number of mechanisms have been proffered by which mitochondrial supply of ATP can change to match varying workload in the myocardium, identifying the underlying regulatory pathways remains controversial.

We describe an approach to studying this problem in which thermodynamically consistent mathematical models of the key energy-consuming processes in the cardiomyocyte (sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA)[1], sodium pump[2] and the acto-myosin cross-bridge cycle[3]) are coupled to a model of mitochondrial ATP production within a whole-cell modelling framework for cardiac excitation-contraction coupling[4].

We use the model to investigate the metabolic stability hypothesis, wherein energy demand-supply homeostasis is maintained despite negligible variation in metabolite concentrations at varying cardiac workloads. We find that under physiological workloads cellular metabolite concentrations do not change significantly with increasing workload if a proposed feedback of inorganic phosphate onto mitochondrial oxidative phosphorylation is present, consistent with the proposition that Pi-regulation alone is sufficient to maintain metabolic homeostasis in the absence of other regulatory mechanisms.

Finally, we use our model to address the empirically observed linearity of the cardiac ATP vs. Force-Length-Area curve (the cellular equivalent of the VO2 vs. Pressure-Volume-Area relationship). We show that the apparent linearity arises from the near irreversibility of the cross-bridge cycle, but that the linear relationship may disappear at cardiac workloads high enough that cellular metabolite concentrations start to vary.

[1] Biophysical Journal 96, 2029-2042, 2009
[2] American Journal of Physiology, Heart and Circulatory Physiology 293, H3036-H3045, 2007
[3] Biophysical Journal 98, 267-276, 2010
[4] Biophysical Journal 90, 3074-3090, 2006

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Stimulation of Cardiac Neoangiogenesis by Estrogen Therapy is One of the Key Mechanisms in Reversing Advanced Heart Failure

Andrea Iorga, Rod Partow-Navid, Humann Matori, Rangarajan Nadadur, Jingyuan Li, Soban Umar, Mansoureh Eghbali.

Estrogen(E2) has been shown to regulate angiogenesis in different tissues, but it is still not known if E2 could stimulate angiogenesis in the heart. Recently, we showed that E2 rescues advanced heart failure by reversing the myocardial contractile deficiency and restoring ejection fraction from ~30% to ~55%. Here we examined whether stimulation of angiogenesis in the heart is a mechanism involved in the E2-induced rescue of HF. Trans-aortic constriction(TAC) procedure was used to induce HF. Once the ejection fraction(EF) reached ~30%, one group of mice was sacrificed and the other two groups were treated with E2(30 μg/kg/day, n=16), or E2 plus the angiogenesis inhibitor TNP-470 (TNP, 30 mg/kg, n=4) for 10 days. Serial echocardiography, real-time PCR and immunocytochemistry were performed. RT-PCR showed that the transcript levels of two markers of angiogenesis, vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1a(HIF1a), were ~10 fold downregulated in HF(0.17 ± 0.06 for VEGF and 0.26 ± 0.01 for HiF1a; normalized to CTRL). E2 treatment was not only able to reverse VEGF and HIF1a transcript level downregulation observed in HF, but to even upregulate both transcripts 3 fold higher than in healthy controls(3.24 ± 0.1 for VEGF and 3.16 ± 0.09 for HIF1a). Quantification of capillary density also revealed that E2 therapy not only completely reversed the loss of capillaries in HF, but significantly enhanced capillary density by ~ 4 fold compared to HF(2.83 ± 0.14 in E2 vs. 0.66 ± 0.07 in HF, normalized to CTRL). Interestingly, E2 failed to rescue HF in the presence of TNP-470 (E2+TNP group) as EF (29.3 ± 2.1%) was not significantly improved after 10 days of therapy. The capillary density of HF mice also did not improve in E2+TNP group(0.53 ± 0.07). These data strongly support the vital role of angiogenesis in the rescue action of E2.

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Estrogen Receptor Beta, but Not Alpha, is the Key Player in Restoration of Heart Function of Heart Failure Mice by Estrogen Therapy

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Recently we discovered that estrogen(E2) therapy can rescue advanced heart failure(HF) induced by pressure overload in mice. Most of the biological actions of estrogen are mediated thorough estrogen receptors alpha(ERa) and beta(ERb), and both of these receptors are present in the heart. Here we investigated which estrogen receptor(s) are involved in the rescue by estrogen. We used the transaortic constriction(TAC) procedure to induce HF. Once the ejection fraction(EF) reached ~30%, one group of animals was sacrificed(HF group), and the other three groups received either 17b-estradiol (30 ug/kg/day), selective ERa agonist (PPT, 0.625mg/kg/day), or selective ERb agonist (DPN, 0.625mg/kg/day) for 10 days. Serial echocardiography was performed and LV pressure was measured by direct catheterization before sacrifice. As expected, E2 rescued HF by restoring EF from 33.17 ± 1.12% to 53.05 ± 1.29%. Interestingly, mice treated with ERb agonist had a significant improvement in their EF from 33.17 ± 1.12% to 45.25 ± 2.1%(n=7), whereas the EF of mice treated with ERa agonist did not improve at all(31.09 ± 2.3%, n=6). Similar to EF, only fractional shortening of DPN-treated mice improved from 15.7 ± 0.58% in HF to 21.95 ± 1.65% in DPN vs. 14.72 ± 1.24% in PPT). Next, we examined the mechanical performance of the LV in mice treated